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Tuberculosis Vaccines and Prevention of Infection

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SUMMARY

Tuberculosis (TB) is a leading cause of death worldwide despite the availability of effective chemotherapy for over 60 years. Although *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) vaccination protects against active TB disease in some populations, its efficacy is suboptimal. Development of an effective TB vaccine is a top global priority that has been hampered by an incomplete understanding of protective immunity to TB. Thus far, preventing TB disease, rather than infection, has been the primary target for vaccine development. Several areas of research highlight the importance of including preinfection vaccines in the development pipeline. First, epidemiology and mathematical modeling studies indicate that a preinfection vaccine would have a high population-level impact for control of TB disease. Second, immunology studies support the rationale for targeting prevention of infection, with evidence that host responses may be more effective during acute infection than during chronic infection. Third, natural history studies indicate that resistance to TB infection occurs in a small percentage of the population. Fourth, case-control studies of BCG indicate that it may provide protection from infection. Fifth, prevention-of-infection trials would have smaller sample sizes and a shorter duration than disease prevention trials and would enable opportunities to search for correlates of immunity as well as serve as a criterion for selecting a vaccine product for testing in a larger TB disease prevention trial. To-

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gether, these points support expanding the focus of TB vaccine development efforts to include prevention of infection as a primary goal along with vaccines or other interventions that reduce the rate of transmission and reactivation.

INTRODUCTION

Mycobacterium tuberculosis infects approximately one-third of humanity and is a leading infectious cause of mortality in the world (1–3). Obstacles to the control of tuberculosis (TB) include difficulties and delays in diagnosis, lengthy treatment regimens, drug resistance, the lack of a highly efficacious vaccine, and an incomplete understanding of what controls transmission, infectivity, reactivation, and progression of disease (3). Although vaccination with *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) protects against TB disease and mortality in some populations, its efficacy is suboptimal and clearly not adequate for disease control (4–8). Developing a more effective vaccine is a high worldwide priority. Investments toward this goal are being made through several approaches, including research leading to a more thorough understanding of the host response to infection, improvement of preclinical models, and a substantial increase in human clinical trial evaluations of candidate vaccines (9, 10).

RATIONALE FOR A PREINFECTION VACCINE

Clinical development of an efficacious TB vaccine requires several choices, including clinical goals (to prevent infection, prevent progression from latent to active disease, or shorten duration of drug treatment), target age, immune status (HIV positive versus negative), geographic location (settings with low, medium, or high endemicity), regimens (replace or boost BCG), platforms (whole cell, viral vector, or adjuvanted proteins), and antigens (RD1 associated, constitutive, or dormancy associated). There are currently 14 vaccine candidates in phase I or II clinical trials (Table 1), and they are largely focused on preventing the development of active TB disease rather than preventing infection (11). Why is there a gap in the development of a TB vaccine that prevents infection? Several factors may contribute to this gap. First, some believe that a vaccine that prevents disease rather than infection would have a higher impact for public health control of TB. Second, there is a perception that the immune system cannot prevent *M. tuberculosis* infection. Finally, due to inadequacies of current animal models to evaluate infection as an endpoint, preclinical data for the majority of candidate vaccines do not sufficiently support advancement to clinical testing for infection prevention.

In this article, we present a multifaceted argument on the merits of pursuing a preinfection vaccine, that is, a vaccine which is developed to prevent infection predominantly in people who have not been previously exposed to *M. tuberculosis*. We begin with the epidemiological data that underscore the potential population benefits of targeting a vaccine to prevent infection. We then highlight biological and immunological steps in pathogenesis that are amenable to early-stage vaccine development. We subsequently review lessons learned from natural history studies which suggest that humans and animals exhibit partial protection from TB infection. We also examine the evidence on BCG and its ability to prevent TB infection. Finally, we use mathematical modeling to assess the plausibility of developing a preexposure vaccine. In addition to the opportunities in this area, there are challenges, including selection of vaccine products, endpoint assays, endpoint definitions, sample sizes, and target populations. Together, these lines of evidence support shifting current priorities to include prevention of infection as a primary goal in the development of a TB vaccine.

TB EPIDEMIOLOGY IN HIGH-PREVALENCE SETTINGS

The recently reported Global Burden of Disease Study documents the ongoing worldwide impact of the TB epidemic (12). TB is the second-highest infectious cause of death worldwide, with 1,196,000 deaths in 2010 (12). The leading cause was HIV infection (1,465,000 deaths), with a substantial proportion of those deaths due to TB. The burden of TB disease is unevenly distributed worldwide, with South Africa, India, and China reporting the highest numbers of cases. Although recent data suggest that TB incidence has decreased in the African region, the estimated annual incidence rates remained over 255/100,000 in 2012 (13). With these high rates of TB disease, efforts to reduce infection could be critical components of an intervention that decreases the TB morbidity and mortality. Development of a preinfection vaccine requires choosing the age for vaccination, suitability for a range of settings with different exposure intensities, and efficacy in HIV-positive and -negative hosts. In this section, we describe the epidemiological data that inform these decisions.

Age-Specific Prevalence and Incidence of TB Infection and Disease

What is the ideal age to administer a preinfection vaccine? With a highly immunogenic vaccine that induces long-term memory responses, administration at birth offers advantages such as opportunities to deliver high rates of vaccination. One disadvantage is the immature immune system of the infant. For a preinfection vaccine, are there epidemiological data to support a rationale for administering the vaccine at a specific age? This answer is related to age-specific incidence and prevalence rates and whether there are any ages where humans are "relatively protected" from TB and may have a higher likelihood of developing a protective immune response. Tuberculin skin testing (TST) has been the most widely used of all the immunological tests for estimation of the prevalence, incidence, and trend of *M. tuberculosis* infection in populations, despite concerns over its sensitivity and specificity (14). In a recently reported study conducted in Cape Town, South Africa, rapidly increasing prevalence of TST responses was seen in healthy HIV-negative township residents between 5 years and 40 years of age (15). Using a cutoff of a ≥10-mm diameter of induration as evidence of latent TB infection (LTBI), almost a fifth of children at school entry were already infected. By the average age of sexual debut at 15 years, 50% of adolescents in these communities were infected (16, 17). By the age of 25 years when HIV prevalence peaks in South Africa, approximately 75% of individuals had evidence of LTBI (15, 18). Between the ages of 5 years and 15 years, the mean annual risk of TB infection remained exceptionally high (range, 3.9% to 4.8%), while the force of infection (the risk of infection in the residual pool of uninfected individuals) was maximal at 7.8% at the age of 15 years (15, 19). The maximal risk of acquisition in the mid-teenage years may reflect social mixing patterns and associated TB exposure in this age group (15, 20, 21). The annual risk of TB infection is so high that individuals may be recurrently exposed to *M. tuberculosis* (22, 23). Recurrent infection may result in infection with multiple strains and could po-

TABLE 1 Candidate TB vaccines in clinical development

Vaccine	Description	Antigen(s)	Adjuvant (receptor)	Phase	Sponsor(s)	Reference(s)
Whole cell						
VPM1002	Recombinant BCG expressing listeriolysin and with urease deleted	BCG		I	Vakzine Projekt Management GmbH, Max Planck Institute for Infection Biology, TuBerculosis Vaccine Initiative, Serum Institute of India	177, 178
RUTI	Fragmented *M. tuberculosis*, immunotherapeutic	*M. tuberculosis*		IIa	Archivel Farma	179, 180
M. vaccae	Whole-cell *M. vaccae*, immunotherapeutic	*M. vaccae*		III pending	AnHui Longcom	181
M. indicus pranii	Whole-cell *M. indicus pranii*, immunotherapeutic	*M. indicus pranii*			Department of Biotechnology (Government of India), Cadila Pharmaceuticals	182
MTBVAC	Viable genetically attenuated *M. tuberculosis*	*M. tuberculosis*		I	University of Zaragoza, Biofabri, TuBerculosis Vaccine Initiative	183
Dar-901	Whole-cell *M. vaccae*	*M. vaccae*		I pending	Dartmouth College	184, 185
Viral vectors						
MVA85A/AERAS-485	Modified vaccine Ankara expressing Ag85A	Rv3804 (Ag85A)		IIb	Oxford University, Aeras	87, 186, 187
Crucell Ad35/AERAS-402	Adenovirus 35 expressing Ag85A, 85B, and TB10.4	Rv3804 (Ag85A), Rv1886 (Ag85B), Rv0288 (TB10.4)		II	Aeras, Crucell	188
Ad5Ag85A	Adenovirus type 5 expressing Ag85A	Rv3804 (Ag85A)		I	McMaster University, CanSino	189, 190
Subunit						
M72	Subunit fusion protein vaccine with Rv1196 and Rv0125	Rv1196, Rv0125	AS01 (TLR4)	IIb	Glaxo SmithKline	191, 192
			AS02 (TLR4)	II	Glaxo SmithKline	
Hybrid 1	Subunit fusion protein vaccine with Ag85B and ESAT6	Rv1886 (Ag85B), Rv3875 (ESAT6)	IC31 (TLR9)	II	Statens Serum Institut, TuBerculosis Vaccine Initiative	193, 194
			CAF01 (Mincle)	II	Statens Serum Institut, TuBerculosis Vaccine Initiative	
Hybrid 4/Aeras-404	Subunit fusion protein vaccine with Ag85B and TB10.4	Rv1886 (Ag85B), Rv0288 (TB10.4)	IC31 (TLR9)	I	Sanofi Pasteur, Aeras	195–197
Hybrid 56/Aeras-456	Subunit fusion protein vaccine with Ag85B, ESAT-6, and a latency antigen (Rv3875)	Rv1886 (Ag85B), Rv3875 (ESAT6), Rv2660	IC31 (TLR9)	IIa	Statens Serum Institut, Aeras	195, 198, 199
ID93	Subunit fusion protein vaccine with 4 *M. tuberculosis* proteins expressed during active and latent disease	Rv2608, Rv3619, Rv3620, Rv1913	GLA-SE (?)	I	Infectious Disease Research Institute, Aeras	200–202

tentially explain the high rates of TB recurrence in individuals receiving curative TB treatment (15, 24, 25). Together, these data suggest that a preinfection vaccine will need to be administered by early adolescence, before most individuals have become infected.

Non-HIV-associated TB disease varies markedly between age strata. In Cape Town, 3 distinct peaks in TB incidence have been observed. The first peak occurs before 4 years of age. Childhood TB disease notifications rapidly decrease after the age of 5 years to a nadir between 10 and 14 years. This decline in TB disease occurs despite a high continuing annual TB infection rate (19, 22, 23, 26), a phenomenon that has been widely recognized but is poorly understood (15, 27–29). There are similarities with the age distribution of meningococcal disease, which has a nadir between the ages of 7 and 14 years (30), followed by an adolescent peak that may be influenced by social mixing patterns but which is also characterized by more clonal genotypes affecting adolescents. A strikingly similar prepubescent nadir in cutaneous leishmaniasis caused by several species of the protozoan parasite *Leishmania* may support an immunological etiology (31). Human resistance to cutaneous leishmaniasis is dependent on Th1 responses (32). While it may be speculated that this has an immunological etiology, further research to understand this phenomenon is warranted. If there is an immune etiology to the relative protection against developing TB disease in this age group, administering a vaccine in this time window may offer benefit.

TB notification rates rapidly increase from the nadir at 10 to 14 years to a second peak between 20 and 24 years. As TB disease is more frequent soon after infection (27, 33–36), this rapidly increasing incidence is consistent with very high infection rates (4.5 to 7% per annum) reported among adolescents in Cape Town (15, 17, 19, 23) and up to 16% in some areas (M. Hatherill, personal communication). These first two peaks have occurred throughout 100 years of TB notification data for Cape Town, and this second peak represents a consistent and ongoing contribution to the TB epidemic in numbers of notified cases.

Notification rates continue to be elevated for older age groups and consist of almost equal proportions of new and recurrent TB disease. Recurrent disease in Cape Town and other high-burden settings has been reported to result predominantly from reinfection (37–39). Multiple reinfections are to be predicted when the prevailing force of TB infection exceeds 1% per annum (15). The total TB burden due to retreatment disease in Cape Town was greater (26%) than that reported for South Africa (18.8%) or the African continent (9.9%) (15, 40), and this may be related to the high prevailing force of TB infection rather than to inadequate or poor case management. Vaccines targeting prevention of reinfection may be an additional feasible strategy to pursue.

HIV-TB Coinfection

The high force of infection in adolescence before the acquisition of HIV infection may be a key factor underlying the explosive HIV-associated TB epidemic in South Africa (17, 41). HIV prevalence among 20- to 39-year-olds in these communities reached 30% in 2002, and the current data suggest that approximately two-thirds of these individuals were likely to have already been infected with *M. tuberculosis* prior to HIV acquisition. Thus, preexisting *M. tuberculosis* infection may be fuelling the high rates of HIV-associated TB in southern Africa (15, 18).

In recent years, there has been a concerted effort to increase provider-initiated HIV testing within the TB service in South Africa (42, 43). In 2009, HIV status was determined for >90% of all TB notifications in the city of Cape Town, enabling stratification of the majority of TB cases by HIV infection status (44). The TB notifications from this city were more than twice the combined annual caseloads of the United States and Canada (40), and rates of both HIV-associated and non-HIV-associated TB were extremely high. The estimated lifetime TB disease risk of 22% was approximately double that observed in studies of TB acquired during childhood in the United Kingdom in the 1950s (27, 33) and was similar to estimates of cumulative TB disease risk in Europe in the early 20th century, prior to the advent of chemotherapy (33, 34).

HIV-infected individuals had a 17-fold-increased risk of TB compared with HIV-negative peers, and the burden of HIV-positive TB closely mirrored the prevalence of HIV infection in the city. Interpretation of age-specific incidence is more complex in the HIV-positive population, as age is only indirectly related to time from acquisition of HIV infection and access to antiretroviral therapy (ART) is an increasing confounder.

The long-term aim of TB control is to lower infection rates in successive generations. The present facility-based TB control is failing to decrease TB infection rates in children and adolescents in South Africa. Systematic evaluation and reduction of infection rates in these high-burden communities should be incorporated as a target of TB control. The development of an effective preinfection vaccine could be a critical component of such efforts.

BIOLOGICAL AND IMMUNOLOGICAL INTERVENTION POINTS FROM EXPOSURE TO INFECTION

Are there immunological reasons to develop a preinfection TB vaccine? The answers to this question are related to the pathogenesis steps that occur upon exposure to *M. tuberculosis* and establishment of infection. Which of these steps are plausible intervention points for a vaccine? From recognition to killing, the macrophage plays a central role in *M. tuberculosis* pathogenesis. First, the bacilli bind to receptors on macrophages and other myeloid cells (monocytes, dendritic cells, and neutrophils) in lungs, where they are detected by the innate immune system (45, 46). Several phagocyte receptors mediate detection of *M. tuberculosis*, including Toll-like receptors (TLRs) (TLR1, -2, -6, -8, and -9), Nod-like receptors (NLRs) (NOD2), and C-type lectin receptors (CLRs) (CLEC4E [Mincle], mannose receptor [MR], and dendritic cell-specific intracellular adhesion molecule-3 [ICAM-3]-grabbing nonintegrin [DC-SIGN and CD209]), and DNA sensors (STING) (47–56). After binding and recognition by innate immune receptors, inflammatory molecules such as tumor necrosis factor (TNF), interleukin-6 (IL-6), IL-10, IL-1β, and IL-12 are secreted. *M. tuberculosis* enters a phagocytic vacuole and usually arrests maturation of the phagolysosome where it resides, often for an extended period of time. The macrophage has several pathways that can kill or limit *M. tuberculosis* replication, including synthesis of molecules with direct antimicrobial activity (e.g., reactive nitrogen intermediates, reactive oxygen intermediates, and antimicrobial peptides), activation of autophagy, and apoptotic cell death (as opposed to necrotic death, which favors *M. tuberculosis* replication and spread to neighboring cells). In the classical model of a successful host response, T cells produce gamma interferon (IFN-γ), which activates macrophages to kill *M. tuberculosis* (57). Although *in vitro* and *in vivo* animal model evidence supports the importance of IFN-γ in this process, the components of

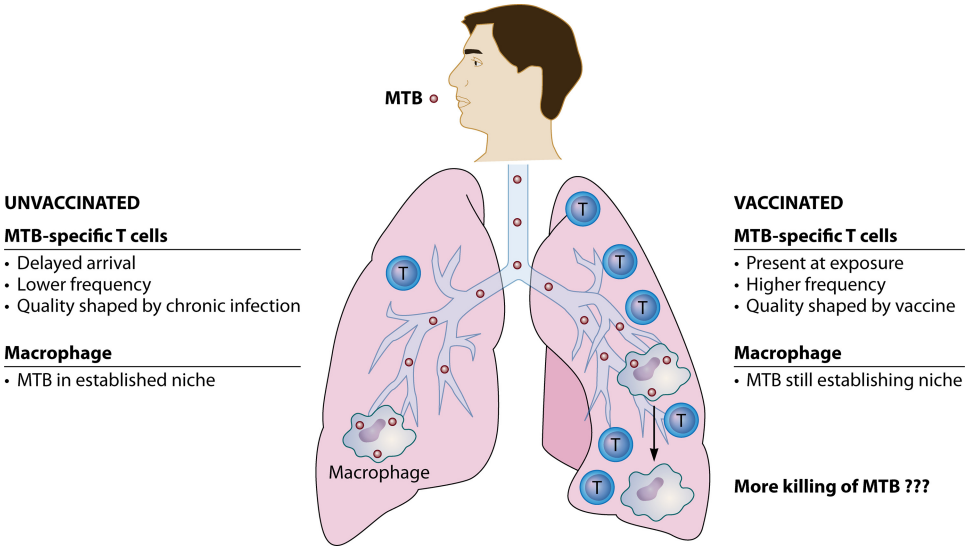

FIG 1 Biological rationale for efficacy of a preinfection TB vaccine. The theoretical benefits of a preinfection vaccine in human lungs after exposure to *M. tuberculosis* (MTB) are depicted. In an unvaccinated individual, development of *M. tuberculosis*-specific T cells is delayed in comparison to that for other infections. During latent infection, *M. tuberculosis* establishes a persistent niche in humans in a location and metabolic state that is poorly understood. Vaccinated individuals could have *M. tuberculosis*-specific T cells available at high frequency during the time of exposure, with the potential to activate macrophages to kill *M. tuberculosis* while it is still metabolically active and before it establishes a persistent infection. In addition, the quality of the T-cell response may be different when shaped by chronic infection versus vaccination. T, *M. tuberculosis*-specific T cell.

a successful immune response involve more than IFN-γ and are largely unknown (58, 59). For example, in a BCG vaccine trial in infants, frequencies of BCG-specific IFN-γ CD4 or CD8 T cells were not associated with risk of TB disease. These findings highlight the importance of discovering and distinguishing what are necessary versus sufficient conditions for protection (60). Activated macrophages and other host cells (T cells, B cells, and fibroblasts) surround the *M. tuberculosis*-infected cells in an organized display, a granuloma, creating hypoxic, acidic, nutrient-poor conditions that are less permissive for *M. tuberculosis* replication. However, the bacilli are not always eradicated. Instead, some survivors adopt a nonreplicating state and can persist for many years, until HIV infection or other factors restore conditions permissive for active replication and potential to progress to active TB disease.

Why is *M. tuberculosis* not eradicated by the innate and adaptive immune responses? Although a robust innate immune response is stimulated upon recognition of *M. tuberculosis* by macrophages, the bacillus employs several mechanisms to promote its survival, including phagolysosome modification, inhibition of apoptosis (and promotion of bacillus survival), and inducing trafficking of cells to the granuloma as a means to expand the number of cells available for infection to increase its survival (46, 52, 61–69). At the adaptive level, early T-cell responses to *M. tuberculosis* are delayed compared to those to other pathogens (46, 70, 71), including influenza virus (e.g., the murine T-cell response is within 1 week for influenza virus versus 2 weeks for *M. tuberculosis*) (72). After humans are exposed, a positive TST does not develop for approximately 6 weeks (73, 74). A similar delay in T-cell immune responses is observed in mice, and this has been associated with delayed transport of bacilli from the lung to the draining lymph node (70, 75, 76). A second feature of adaptive immune responses to *M. tuberculosis* is that antigen-specific T cells often fail to recognize and eradicate *M. tuberculosis*-infected macrophages (46, 77). Several mechanisms have been linked to impaired *M. tuberculosis* killing, including a protected cellular location for *M. tuberculosis* during the first week that prevents recognition and killing (78), inhibition of IFN-γ-activated pathways in macrophages (79–81), and the development of regulatory T cells (82). In summary, *M. tuberculosis* employs multiple mechanisms to inhibit innate and adaptive immune responses and to establish a chronic persistent infection.

The primary focus of TB vaccine efforts thus far has been to prevent cases of active TB disease. Shifting the goal to preventing infection offers several advantages for countering the immune evasion strategies of *M. tuberculosis* (Fig. 1). First, the delayed development of $CD4^+$ T-cell responses after *M. tuberculosis* exposure provides a protected-time window of 6 weeks for the bacillus to establish infection. If $CD4^+$ T-cell or other effector cell responses were present at the time of exposure, a major feature of early *M. tuberculosis* evasion strategies could be circumvented. Second, the kinetics of killing *M. tuberculosis*-infected macrophages may also be fundamentally different in acute versus chronically infected cells. If T cells produced IFN-γ to activate macrophages immediately after infection, clearance of the bacillus may be more likely due to less time for development of *M. tuberculosis*-directed evasion strategies. Nevertheless, some of the immune evasion strategies would likely present challenges for vaccines directed at preventing infection. First, the influence of regulatory T cells on vaccine efficacy (VE) would likely affect all stages of infection. Interestingly, many vaccine efficacy studies use laboratory-adapted strains that induce minimal Treg responses in murine models in comparison to those induced by more-virulent clinical strains such as HN878 (83). Second, the potential existence of a "protected" location for *M. tuberculosis* during the first week of infection poses a challenge even if T cells are present at the time of exposure. The features and location of this potential protected area are not known (e.g., lymph node versus alveolar space versus

interstitium, cell type, and intracellular versus extracellular). This second concern arose from a murine study testing this concept in which ESAT-6-specific $CD4^+$ T cells were adoptively transferred into naive mice before aerosol challenge with *M. tuberculosis* (78). Despite the presence of high levels of TH1 effector cells in the lungs, no protective effect in comparison to control mice was observed until 7 days postchallenge. Third, the quality and magnitude of a protective vaccine-induced memory T-cell response may be different from those of the response generated by primary infection. One study compared the effects of primary (first exposure) and secondary (generated by infecting mice with H37Rv for 30 days and then treating the infection with isoniazid [INH] and rifampin) T-cell responses on control of *M. tuberculosis* replication (84). Although the mice with a secondary immune response had significantly lower lung *M. tuberculosis* burdens than naive mice after *M. tuberculosis* aerosol rechallenge, there was no evidence of sterilizing immunity or prevention of infection. These data suggest that the mere presence of *M. tuberculosis*-specific T cells at the time of exposure is not sufficient to prevent reinfection. However, if the T cells were qualitatively different, not directed against one antigen, and present in higher quantities in the lung at the time of exposure, protection may be possible. Efforts to measure the quality, quantity, and location of *M. tuberculosis*-specific T cells postvaccination may elucidate mechanisms of protection in human trials (e.g., measuring activated effector versus central memory versus effector memory CD4 T cells) (85, 86). Understanding why TH1 effector T cells are ineffective during the early phases of infection and identification of efficacious qualities (or mechanisms of inhibition of efficacy such as contraction, exhaustion, or Treg induction) will be critical for successful development of a preinfection vaccine.

IS REDUCING TB ACQUISITION RISK BY VACCINATION PLAUSIBLE?

Lessons from Natural History Studies

Current TB vaccines in clinical development are focused on developing T-cell responses to secreted immunodominant antigens (Table 1) (10). Both infants and adults have been targeted in clinical trials thus far, with primary endpoints that include incidence of active TB disease as well as mortality from TB (9, 87, 88). Is there biological data available to address the plausibility of a vaccine that protects against TB infection? Vaccine-induced prevention of infection would likely manifest as an adaptive response capable of clearing the pathogen and thus preventing the establishment of persistent infection (either completely preventing initial macrophage infection or rapidly killing/sterilizing the infected macrophage). We present three arguments that prevention of infection is a feasible goal for TB vaccines. The data supporting these arguments come from animal model studies, human natural history cohorts, and BCG vaccine trials.

Natural history studies: animal models. A natural TB infection animal model offers the potential to address several difficult questions about the transmissibility of *M. tuberculosis* as well as mechanisms of resistance to infection and/or progression to active disease. The guinea pig is highly and uniformly susceptible to *M. tuberculosis* infection and disease under laboratory conditions of infection (89, 90). In contrast, under natural exposure conditions, guinea pigs demonstrate a range of susceptibility that is similar to that of humans. Riley et al. designed the original natural exposure experiments in the 1950s with shunting of air from hospital rooms of humans with pulmonary TB into separate rooms where guinea pigs were housed (91–93). Guinea pigs had serial TST skin tests during the experiment with necropsy at the conclusion and culturing tissue for viable *M. tuberculosis*. Some infected animals with positive TSTs reverted their skin test and had no evidence of active TB disease at necropsy (91, 92). These data suggested that reversion was associated with sterilizing immunity. An alternative interpretation of these data is that the initial TST conversion was due to exposure to dead bacilli that did not produce a true infection. Subsequent studies assessed this possibility by exposing the ward air to UV light and demonstrating that killed *M. tuberculosis* did not induce conversion to a positive TST (94).

More recently, Dharmadhikari et al. extended these studies and examined transmissibility and disease progression in guinea pigs naturally exposed to multidrug-resistant (MDR) *M. tuberculosis* (93). Serial TST skin testing was done at baseline and throughout 20 weeks of exposure to assess whether TST magnitude and kinetics were associated with disease progression. Among 362 guinea pigs exposed for 4 months to patients with MDR TB, 75% of animals had positive TSTs (>6 mm) (93). Interestingly, only 12% of the animals with a positive TST had active TB when assessed at necropsy by histology and culture of lung, spleen, and lymph node. To determine whether early or late TST conversions were predictive of different outcomes, animals with large TSTs (>14 mm) at 20 weeks were analyzed further. Those with early conversions had lower disease severity than those with later conversions. To further assess the kinetics of TST conversion, those with positive tests in the 6- to 13-mm range were analyzed. Twenty-two percent ($n = 86$) of these animals had a reversion of their test to negative, and only 2/86 had active disease. In contrast, 47% of animals with nonreverting skin tests had active disease. Even after steroid administration to a subset of animals, there was no evidence of active disease in the animals exhibiting TST reversion. A potential confounding variable is that serial skin testing could induce or boost cell-mediated immunity. Although this is possible, other investigators reported only rare false-positive conversions in control animals without exposure to *M. tuberculosis* that received serial skin tests (95). Together, these experiments demonstrate that natural exposure causes a more diverse set of outcomes (resistance, reversion, and range of disease severity) than laboratory conditions. Some of these differences may be due to the metabolic state of the bacillus or the magnitude of the exposure. In addition, these experiments suggest that a guinea pig can completely clear *M. tuberculosis* infection and that reversion of a TST was correlated with this possible sterilizing immune response. These studies are promising and provide an impetus to understand whether immune responses that can clear an established infection exist in humans. In addition, the difference in outcomes between natural and laboratory-controlled exposure suggests that vaccine trials in animal models may need to incorporate such conditions in their design to avoid artifacts of laboratory testing conditions.

Natural history studies: humans with resistance to TB infection. Are any humans resistant to TB infection? Although many individuals become infected with *M. tuberculosis* after sustained exposure, only a small fraction resist infection as demonstrated by persistently negative TSTs or IFN-γ release assays (IGRAs) (96, 97). These individuals may have an innate macrophage response to *M. tuberculosis* that resists initial infection or rapidly clears the

bacillus before a T-cell response develops. If such innate resistance did not exist, one might expect LTBI rates in regions where TB is endemic to approach 100%. Resistance is likely a threshold phenomenon that is related to the degree of exposure. Surprisingly, approximately 5 to 10% of individuals remain tuberculin skin test negative despite sustained exposure to an infectious TB case (96). Addressing this question experimentally requires a longitudinal study design with documented events of exposure to individuals with pulmonary TB, ideally with high bacillary loads in a household where there is extensive exposure with contacts. Since 1995, the National Institute of Allergy and Infectious Disease (NIAID)-supported Tuberculosis Research Unit (TBRU) conducted a household contact (HHC) study in Kampala, Uganda (98–105). Index cases were identified at the Uganda National Tuberculosis and Leprosy Program treatment center at Old Mulago Hospital in Kampala, Uganda. Participants were enrolled if they were 18 years or older, had sputum smear-positive and/or culture-positive pulmonary TB, and had at least one HHC living with them. Individuals whose TSTs were negative at all follow-up visits were considered to be resisters (RSTR), and individuals who developed a positive TST during study follow-up were considered TST converters (96, 97). All of the individuals in the HHC study live with the index case and have many risk factors for close proximity to the index case. Extensive epidemiological analysis has not revealed any exposure variables that distinguish those who convert their TST from those who do not. A potential confounder of the study design is that *M. tuberculosis*-specific T cells may be present in other tissues (e.g., skin or lung) and not detected by skin testing or peripheral blood assays. A whole-genome linkage scan and candidate gene study was performed to identify human genetic risk factors for resistance to TB infection (RSTR) and active TB disease. In the genome scan, regions linked to active TB differed from those linked to RSTR. Linkage results for RSTR included a chromosome 5 region that has since been replicated by an independent study (106). Although not conclusive, these data suggest that some humans are naturally resistant to TB infection and that genetic factors may regulate this difference. Identification of the immunogenetic factors associated with resistance may lead to novel strategies for immunomodulatory therapy. These strategies might include the use of small-molecule drugs that target host macrophage pathways or vaccines that induce T-cell responses which activate macrophages to kill *M. tuberculosis* (107).

Natural history studies in humans with preexisting TB infection: is there evidence of protection? Is there evidence of acquired immunity to TB from natural history studies? This question has been partially addressed by studies in regions of high endemicity that compare active TB incidence rates in those with or without TB infection. A high-endemicity setting is required to assess this question since there is an assumption of ongoing exposure to *M. tuberculosis* during the study period. The classic study to address this question was performed by Johannes Heimbeck in Norway, who administered TSTs to nursing students who entered school between 1924 and 1936 and worked in a hospital with a large number of TB patients (108). Among 1,453 students, 45.3% were TST positive at study entry and 54.7% were negative. Within the TST-negative group who did not receive BCG vaccination (n = 284), 34.2% developed active TB disease and 10 died. In contrast, 3.3% (n = 22) of the TST-positive nurses developed disease, with no deaths. Multiple studies subsequently examined this question with prospective cohort designs in the prechemotherapy era. In a meta-analysis of 18 studies with an aggregate sample size of 19,886 individuals, Andrews et al. estimated that individuals with latent tuberculosis had a 79% lower risk of developing active TB disease after reinfection than uninfected individuals (109). In a more recent study in a region in South Africa with a high TB burden, 6,363 adolescents 12 to 18 years old were followed for 2 years with serial TST and QuantiFERON testing (QFT) (110, 111). Among those who had a positive baseline QFT, the incidence rate was 0.64 active TB cases/100 person years. In contrast, the rate among those who converted during the 2-year observation period was 1.46 cases/100 person years. In another recent study of 764 households in Peru in an area of high BCG vaccination rates, modeling of cross-sectional data estimated that previous infection reduced the risk of reinfection by 35% compared to that for uninfected individuals (112). Together, these studies suggest that individuals with established *M. tuberculosis* infection have decreased susceptibility to active TB disease after reinfection in comparison to those who are uninfected. These data are consistent with a concept that humans can acquire immunity to TB from natural exposure.

Natural history studies: spontaneous human reverters. If humans could eradicate *M. tuberculosis* after infection, it would support the rationale that such protective immunity could be induced in a vaccine. Although this immunity would not prevent infection, it could possibly eliminate *M. tuberculosis* in the early stages before it establishes a chronic infection. Is there evidence from natural history studies that such "natural immunity" exists? Although the majority of individuals with a positive TST will remain positive for their lifetime, the phenomenon of reversion has been recognized for over a century (113). Spontaneous reversion (in the absence of INH treatment) may represent a successful host response, and these individuals can be studied to identify correlates of protective immunity. Alternatively, reverters may have suppressed or defective immune responses and still harbor active bacilli that are fully capable of causing active TB disease. Does any epidemiological evidence favor one of these models? Early studies demonstrated that many individuals spontaneously revert from a positive to a negative TST. This was first published in 1913 by Gelien and Hamman in their study of 1,000 individuals in Baltimore, MD, who received a TST in ~1908 and a repeat test 1 to 3 years later (114) (Table 2). Nearly 50% of those subjects reverted their positive test. Many additional studies had similar findings over the next 100 years and explored factors associated with reversion. Consistent findings across multiple studies suggest that reversion is more common in children (115–117), less common in those with a higher-magnitude initial conversion (113, 118), and possibly more common in females (115, 119). Rates of spontaneous reversion may depend on several variables, including BCG vaccination, exposure to nontuberculous mycobacteria (NTM), a booster phenomenon from prior TSTs, duration of positivity before reversion, age, and magnitude of the conversion and reversion (118, 120). To control for some of these variables, recent studies have used *M. tuberculosis* antigen-specific tests and found high rates of reversion for recent converters in analyses that were adjusted for these variables (110, 117, 119). Hill et al. used a CFP10/ESAT-6-based enzyme-linked immunosorbent spot (ELISPOT) assay in the Gambia and reported reversion rates of up to 36% (117). Using the IGRA QuantiFERON-TB Gold (QFT-TB Gold), Shah et al. found a reverter rate of 15% in children in Soweto, South Africa, and Machingaidze et al. found a rate of 9.2% in Worcester, South Africa (110, 119).

TABLE 2 Spontaneous reverters[a]

Study start yr	Location or population	N/n[b]	Age (yr), time interval between tests	% Reversion, test used[c]	Comments	Reference
1913	Baltimore, MD	1,000/?	All, 1–3 yr	50, PPD	Minimal exptl details	114
1924	Philadelphia, PA	3,919/2,490	All, 6 mo	11.1, PPD	Reversion rate inversely correlated with initial conversion magnitude; reversion more common in young than in adults; among 913 clinically active TB patients, none reverted; among 276 reverters, 11 had CXR evidence of healed, calcified TB	116
1935	Native Americans in USA	3,025/?	0–20, not stated	7.8, PPD	No relationship of reversion to mortality (but only 10 died)	203
1959	Los Angeles, CA	160/121	<6, 3–12 mo	56, PPD	Reversion rate inversely correlated with initial conversion magnitude; majority not treated with INH; higher reversion rate if CXR with inactive lesions (10/20); no reversion if CXR had active lesion ($n = 14$)	113
1980	Malawi	6,991/1,889	All, 5 yr	3–15, PPD	Higher reversion in age <5 and in females	115
1982	San Francisco, CA	495/258	Adults mean age 76, 3 yr	24.8, PPD	98% of reverters had 0 mm at second test	204
<1990	San Francisco, CA	380/380	31–105, 1 yr	26, PPD	70% complete reversion; 60% reversion in age >90; lower reversion rate if initial test >15 mm	118
<2006	The Gambia	558/313	>15, 3–18 mo	8.9, PPD; 32.7, ELISPOT	Reversion rate inversely correlated with age; higher reversion for ELISPOT than for PPD	117
2006	Soweto, South Africa	270/62	3–9, 6 mo	15, QFT-GIT	Higher reversion in females; lower reversion if baseline positive PPD	119
2005	Worcester, South Africa	6,363/2,613	12–18, 24 mo	9.2, QFT-GIT		110

[a] The table excludes studies that use isoniazid or other chemotherapy (except for that by Adams et al. [113]).
[b] N is the total number in the study (positive and negative results), and n is the number of individuals with a positive test who were examined for reversion with a second test.
[c] Percent reversion among those with a positive test.

Several reasonable concerns have been raised regarding the interpretation of the significance of reversion. First, some reversions likely represent borderline positive results with fluctuations slightly above and below the positivity threshold. Although this is the case for some reverters, multiple studies have demonstrated that the majority of reverters have large and often complete reversions, with a second test of 0-mm induration after an initial conversion magnitude of 10 to 15 mm. Second, reverters may have transient exposure to nontuberculous mycobacteria rather than true infection with *M. tuberculosis*. More recently, this concern has been fully addressed with three studies that used IGRAs (QFT-TB Gold In-Tube [QFT-GIT] or ELISPOT) and found reversion rates of 8.9 to 15% (110, 117, 119). Third, reversions are a phenomenon found only in low-incidence areas and may represent false-positive tests and/or tests compounded by laboratory errors. This concern is unlikely for multiple reasons, including that the majority of the studies are from countries that currently have high TB endemicity or those that previously had high rates (e.g., the United States in 1900 to 1950). The consistency, magnitude, and frequency of the reverter phenomenon over multiple studies suggest a durable finding.

What does reversion represent immunologically? The reversion of *M. tuberculosis*-specific immune responses could be caused by immune suppression, egress of *M. tuberculosis*-specific T cells from the blood, clearance of TB infection, or lowering of the *M. tuberculosis* bacillary load. Although the TST conversion and reversion event likely includes $CD4^+$ T-cell IFN-γ, there has been no systematic study of the details of the cellular source of IFN-γ or the breadth of other immunological responses and whether they also revert. For example, other immunological responses that may revert include other cytokines (e.g., IL-2, TNF, and other T-cell cytokines), different T-cell subsets (e.g., $CD4^+$ versus $CD8^+$ versus CD1), different $CD4^+$ T-cell phenotypes (e.g., TH1 versus TH2 versus TH17 and central versus effector memory), and responses to other *M. tuberculosis* antigens (beyond CFP10, ESAT-6, and TB7.7 in the IGRA). Reversion of either global or restricted *M. tuberculosis*-specific immune responses could represent immunologically useful signals to dissect protective immune responses.

Do reverters have a lower incidence of TB disease than converters? The most comprehensive assessment of this question was performed by Arthur Dahlstrom with an HHC study of TB index cases in Philadelphia in the 1920s (116). A total of 3,919 individuals from 513 households were followed for at least 5 years with TSTs, chest X rays (CXRs), and clinical assessments. Among 2,828 individuals for whom at least 2 TSTs were performed, 276 (11.1%) reverted their test to negative (<5 mm). Among the 913 (23.3%) of individuals with active TB in this study, none were TST reverters. Among 274 reverters with CXRs obtained at the time of their reversion, 2 had evidence of active childhood TB (presumably without symptoms), while the remainder showed either no TB

($n = 256$) or inactive TB ($n = 16$). Together, these data suggest that reverters have a minimal risk of developing active TB over 5 years (0.72% versus 23.3% of the entire cohort). In addition, the same study as well as others demonstrated that some reverters develop evidence of substantial TB disease that subsequently heals with calcified granulomas (113, 116). Together, these studies support the concept that reverters are individuals who possess protective immune responses to *M. tuberculosis* that could be immunologically interrogated to discover correlates of protective immunity. To our knowledge, such studies have not yet been performed. Although suggestive, these studies were performed without current standards of study design and statistical analysis, and interpretation of some of the data is uncertain. Repeating a longitudinal study of reversion and its clinical outcomes along with immunological interrogation of the response is needed to determine the significance of reversion.

Lessons from BCG

Does BCG provide sterilizing immunity? What data are available from animal and human studies to address this question?

BCG animal studies. Since BCG was first developed, its effects as a vaccine have been investigated in countless experiments in different animals from rodents to rudiments to primates, each with intrinsic limitations and differences. Generally, the majority of these studies employed aerosol routes to deliver *M. tuberculosis* challenge doses that are as low as possible and yet still ensure uniform infection and disease outcome for all animals. Thus, infection doses in experimental animal studies tend to be significantly higher (50 to 3,000 CFU per animal, commonly 10 to 20 for guinea pigs and 100 for mice) than the relatively few bacilli thought to be transmitted during natural exposure (though the precise number has never been measured) (91, 121–123). In addition, the bacillus is prepared under laboratory conditions with a metabolic state and physical conditions (sputum versus broth culture) that likely differ from those in natural transmission settings (93). In this setting, BCG does not promote sterile clearance of *M. tuberculosis* but rather controls *M. tuberculosis* replication to different degrees. In the best-studied murine, macaque, guinea pig, and rabbit models, BCG recall responses have been shown to arrest *M. tuberculosis* growth several days earlier than in primary infection (89, 90, 124–127). Consequently, bacterial burdens and disease severity are reduced and time to death is increased.

(i) Low- versus high-dose challenge models. Does BCG provide greater immunity in very-low-dose challenge models in comparison to those with high doses? Early BCG studies more commonly employed lower challenge doses as a means to more closely mimic natural transmission. However, infection is more difficult to verify with lower *M. tuberculosis* doses, and they are rarely utilized in current studies. The sensitivity of different animals to *M. tuberculosis* also varies. For example, commercial rabbits are relatively resistant to *M. tuberculosis*, requiring approximately 300 to 3,000 inhaled bacilli to produce 1 primary pulmonary lesion, and are therefore less well suited for low-dose infection models (89). Another constraint for small-animal studies utilizing aerosol infection chambers is the potential for partial infection of a group of animals, as has been recently described for aerosol chamber infection of mice at doses lower than 10 CFU per infected animal (122). Guinea pigs are very susceptible to *M. tuberculosis*, generating roughly 1 primary pulmonary lesion per inhaled bacillus, and thus low-dose challenges may be delivered via infection chambers. Using an aerosol challenge of <10 CFU per animal, BCG vaccination has been shown to restrict *M. tuberculosis* growth in the primary pulmonary lesions of guinea pigs (126, 128). Vaccinated groups contained fewer primary lesions and no secondary lesions. In nonvaccinated groups, numerous secondary pulmonary lesions, as well as extrapulmonary lesions, developed as a result of *M. tuberculosis* spreading via the bloodstream, indicating that all animals were infected. In another low-challenge-dose guinea pig study, aerosol delivery of BCG was associated with a profound reduction in bacterial burden that was greater than that observed at higher challenge doses (129). Together, these studies suggest that BCG vaccination prevented more primary lesions than in controls, but it was not 100% effective.

(ii) NHP studies. Macaques are also relatively susceptible to *M. tuberculosis* and thus amenable to low challenge doses. Aerosol infection devices have been used to infect rhesus macaques with doses on the order of 10 to 15 CFU (130, 131). TST reactivity and evidence of disease via radiographic, pathological, and tissue sample culture techniques were used to evaluate *M. tuberculosis* infection outcome in these studies. Using these methods, 9 out of 10 monkeys receiving an aerosol or intravenous BCG vaccination were protected, exhibiting no evidence of *M. tuberculosis* infection at 12 weeks postchallenge (131). Similar findings were observed for a group receiving intravenous BCG (8 out of 10 were protected). However, the fact that 2 of 10 monkeys in the control unvaccinated group lacked evidence of infection suggests heterogeneity in infection. Alternatively, there was heterogeneity in disease outcome as has been described for cynomolgus macaques (132, 133). Intrabronchial or intratracheal instillation has also been used to more directly deliver doses ranging as low as 10 to 25 CFU (132, 134, 135). However, vaccination has thus far not been investigated using these routes together with very low *M. tuberculosis* challenge doses. The BCG studies in nonhuman primates (NHPs) conducted thus far have utilized high challenge doses (250 to 3,000 CFU per animal), and in these studies, reduction in bacterial loads and hematogenous spread, but no evidence of sterilizing immunity, have been observed (136–138). Although the pathological, radiographic, and sample culturing techniques do not definitively demonstrate *M. tuberculosis* clearance, the high inocula utilized are a limitation for interpreting the potential efficacy of BCG in preventing primary infection. Nonetheless, a variety of animal models indicate that the protective effects of BCG may be greater when very low challenge doses are used.

(iii) Ruminant studies: *M. bovis*, cattle, and deer. BCG vaccination has also been evaluated as a means to protect against *M. bovis*, the etiological agent of tuberculosis in domestic livestock and wildlife animal reservoirs. Similar to findings in humans, protective efficacies for BCG against animal tuberculosis have been variable, ranging from no protection to significant protection from disease (139–143).

The variable results from experimental *M. bovis* infection of cattle have led to similar challenges and technical constraints as those described above for *M. tuberculosis*. Although low *M. bovis* challenge doses would better mimic natural transmission, to ensure uniform outcome, typical doses involve 2×10^3 to 5×10^3 CFU given intratracheally. In the majority of studies, protection has been characterized as reductions in *M. bovis*-induced pathology and bacterial loads rather than prevention or clearance of infection (144, 145). Although there is variability within groups, there are reports suggesting that some BCG-vaccinated cattle

lacked evidence of disease, even in the case of high-dose *M. bovis* challenge (146, 147). In studies that reported pathology and culture results for individual cattle, there is evidence that some BCG-vaccinated cattle lacked macroscopic lesions and also had no bacilli cultured from lung or lymph node samples (148, 149). In a recent study of a BCG-containing prime/boost regimen, administering a viral vector boost vaccine improved vaccination outcome and resulted in greater numbers (60%) of cattle that lacked evidence of disease in terms of visible lesions or culturable bacilli (150).

Deer represent one of the wildlife species that serve as a reservoir for *M. bovis* transmission to livestock. In a deer BCG model, 200 to 500 CFU of *M. bovis* inoculated into a tonsular crypt was found to result in infection of >90% of deer and to induce pathology similar to that of natural infection (144). Importantly, in addition to protection against development of disease, the investigators evaluated BCG's ability to protect against infection. In these studies, low doses of BCG and boosting were necessary for preventing infection in all animals, while suboptimal regimens achieved protection from disease only (151, 152). While it remains possible that absolute sterile clearance was not achieved, the absence of culturable bacilli from individual and pooled lymph nodes in 5 out of 5 animals is highly suggestive of clearance (151).

Even more compelling evidence of sterile clearance was reported in a cattle study in which *M. bovis*-specific responses in peripheral blood were evaluated along with pathology and culturing (148). Two vaccinated and challenged cattle in this study lacked evidence of disease and also exhibited a transient ESAT-6-specific IFN-γ peripheral blood response several weeks postchallenge (148). These data are reminiscent of the IGRA response reversion in humans discussed above and could indicate that BCG-induced responses enabled these cattle to clear their *M. bovis* infection.

Does BCG provide sterile clearance of *M. bovis* in natural-exposure settings? Several field studies have observed significant efficacy for BCG in reducing incidence of bovine TB (143, 153, 154). Field studies in Malawi showed that a single subcutaneous BCG dose provided significant protection against lesions (141, 143). More recently, two field studies in Mexico and Ethiopia were conducted in which BCG efficacy was estimated at around 60% (153, 154). These studies employed gamma interferon release assays using ESAT-6 and CFP10 stimulation of whole blood and thus were able to distinguish partially protected from fully protected cattle.

Finally, a field study among badgers, another wildlife reservoir for *M. bovis*, found that BCG vaccination protected against infection (155). In this study, the estimated reduction in infection risk ranged from 54% to 76% depending on the diagnostic test used. In addition, a reduced risk of infection was also observed for unvaccinated badger pups, suggesting a herd immunity effect. Taken together, these *M. bovis* studies demonstrate that BCG protects against development of lesions and other disease parameters. In a few reports there are also indications that BCG-induced immunity may lead to bacillary clearance, particularly for natural-transmission settings (153, 154).

(iv) Is there evidence that BCG can promote *M. tuberculosis* clearance in animal models? Although there are suggestive data in multiple models that it may be possible, definitive evidence of vaccine-induced *M. tuberculosis* clearance is lacking, mostly because of the technical challenges involved in demonstrating infection status. Future studies could solve some of these study design limitations. For example, macaque studies utilizing very-low-dose challenges via aerosol or intrabroncheal instillation, similar to those of Barclay et al. (131), could be conducted in conjunction with early bronchoalveolar lavage sampling for culture as well as IGRAs to verify infection and to assess the possibility of clearance over time. In addition, a recently described ultra-low-dose challenge mouse model could be used to assess the protective effects of vaccination when mice are infected with <10 bacilli (122). Although such low doses result in partial infection, the use of mice, which are relatively low in cost, would permit the necessary increase in animals used per experiment. Natural-exposure studies should also be considered, for example, using the guinea pig clinic ward air exposure approach to evaluate the potential for vaccines to promote sterile clearance (91, 93). Early time points to assess infection rates and extended sampling performed in conjunction with pathogen-specific immune responses (i.e., IGRA) would further strengthen the data. Although such animal models have potential to be used as a screening step for vaccine selection, animal models have intrinsic limitations due to species-specific immune responses that differ from those of humans e.g., potentially different macrophage mechanisms of *M. tuberculosis* killing in humans (antimicrobial peptides) versus mice (nitric oxide).

BCG human studies. In addition to animal data, there are human studies that suggest a possible protective effect of BCG. Prior to the development of IGRAs, this question was confounded by cross-reactivity between purified protein derivative (PPD) and BCG due to common antigens. The antigens used in the T-SPOT ELISPOT assay (CFP10/ESAT-6) and QFT-TB Gold In-Tube assay (CFP10/ESAT-6/TB7.7) are not present in BCG and thus can be used to assess whether BCG vaccination is associated with protection from TB infection. A recent meta-analysis of 14 retrospective case-control studies suggests that BCG is associated with protection from *M. tuberculosis* infection (n = 3,855; overall risk ratio, 0.81; 95% confidence interval [CI], 0.71 to 0.92) (156). Four additional studies were not included in the meta-analysis. Three of those four studies also showed a protective effect (Table 3) (117, 157, 158). One limitation of these studies is that they were retrospective and most relied on the presence of a BCG scar to document vaccination status (except the study from Greenland, which had birth records of BCG vaccination). Several studies that demonstrated a protective association were performed in countries with low or medium incidence (United Kingdom, Europe, Turkey, and Greenland). These data suggest that BCG protection could be dependent on the level of exposure, with protection waning in high-exposure settings. Differences in efficacy that correlate with geography also suggest possible effects from nontuberculous mycobacteria (NTM) which could potentially modulate vaccine responses through heterologous priming from cross-reactive mycobacterial antigens. NTM exposures can vary substantially in different geographic regions, which might explain the disparate outcomes of BCG vaccination in large trials conducted in northern latitudes (e.g., British Medical Research Council trials with 80% efficacy) and equatorial regions (Indian Council of Medical Research Trial in India and Karonga Prevention Trial in Malawi with 0% efficacy) (159–161). Although some immunological data support this hypothesis, the nature, magnitude, and mechanism of this potential modulatory effect are unknown (162, 163). These issues could affect the choice of where to conduct a prevention-of-infection trial. Although these studies are not randomized, pro-

TABLE 3 BCG and protection from TB infection[a]

Study yr	Location	*n*	Age	Odds ratio (95% CI) for BCG vs IGRA	Comments	Reference
2002	Turkey	979	<16 yr	0.60 (0.43–0.83)	ELISPOT; household contact design, 7 clinics in Istanbul	205
2002-2004	The Gambia	718	6 mo–14 yr	0.80 (0.5–1.2)	ELISPOT; household contact design	206
<2007	The Gambia	207	>15 yr	0.50 (0.20–1.0)	ELISPOT; household contact design	117[b]
2007-2008	Australia	524	5 mo–16 yr	1.80 (0.80–4.0)	ELISPOT; refugees from Africa and Burma	207[b]
2006-2009	Europe	1,128	<16 yr	0.41 (0.30–0.55)	QFT-GIT; pTB-NET multicenter, multicountry study	157[b]
				0.41 (0.25–0.66)	ELISPOT; pTB-NET multicenter, multicountry study	
1982-2006	Greenland	953	5–30 yr	0.52 (0.32–0.85)	Assessed TB infection before and after change in nationwide BCG usage	158[b]

[a] Studies with *n* of >700 or not included in meta-analysis by Roy et al. (156).
[b] Study not included in meta-analysis by Roy et al. (156).

spective, consistent, or conclusive, they do suggest that BCG may protect against TB infection. Based on the animal and human studies published to date, an important next step for the field would be to conduct a randomized clinical trial of BCG vaccination for prevention of TB infection.

Lessons from Mathematical Modeling

What other factors influence the plausibility of a preinfection vaccination strategy for TB? If a vaccine did reduce the *M. tuberculosis* acquisition risk, would this translate to observed efficacy in a clinical trial conducted in a high-burden setting? Current models suggest that a neonatal preexposure vaccine could reduce TB disease incidence rates by 39% from 2015 to 2050 (164). However, these models have not assessed the impact of a vaccine that prevents infection. To address this question, we used mathematical modeling to explore how different *M. tuberculosis* exposure/transmission parameters could affect the likelihood of infection and, subsequently, whether a vaccine's apparent efficacy is readily observed or masked.

There is little quantitative information known about the intensity (number of exposures over time) and magnitude (number of bacilli per exposure) of exposure to *M. tuberculosis* and how variations in these are related to risk for infection. Suppose that prior vaccination decreases the probability of a single viable organism (or a single "droplet micronucleus") deposited on the lung alveolar surface establishing a persistent infection. How might variations in the intensity (numbers of exposure events) and magnitude (numbers of bacilli deposited per exposure event) of exposure affect the efficacy to prevent infection induced by a vaccine? Because these early events in the process of exposure and establishment of infection are not observable, one can only address these questions by asking "what if" questions with answers provided by mathematical modeling. The goal of this section is to posit a simple model for intensity and magnitude of exposure to *M. tuberculosis* and to use that model to explore the levels of efficacy that might be expected from a vaccine that reduces the probability of a single bacillus from establishing a persistent infection.

Model for intensity and magnitude of *M. tuberculosis* exposure. We start by assuming that M represents the number of exposure events over a given year of follow-up. Note that we implicitly assume a model of discrete exposure events such as might arise via social contacts rather than a model of continuous exposure such as might arise via constant contact with an infected caregiver. We assume that the distribution of M has expected value μ_M and variance $\sigma^2 M\mu_M$. The parameter μ_M is used as an index of the intensity of exposure over time, and $\sigma^2 M$ is an index of variability over individuals in exposure intensity. We specifically use an overdispersed Poisson (negative binomial) distribution for M to simplify our formal calculations.

Now suppose that for a given exposure event, the number of viable bacilli (or droplet micronuclei) deposited on the lung alveolar surface is represented by X. We assume that the distribution of X is also negative binomial with expected value μX and variance $\sigma^2 X\mu_X$ and that the magnitudes of exposure realized over separate exposure events are independent. The parameter μX is used as an index of the magnitude of exposure over exposure events, and $\sigma^2 N$ is an index of variability in magnitude of exposure events over time. Note that X can more generally be thought of as a surrogate measure of the infectious potential for a given exposure (e.g., due to *M. tuberculosis* strain variation).

The model linking exposure to infection is completed by assuming that given an exposure event of magnitude X, the probability of a persistent *M. tuberculosis* infection is given by the function $P(X{:}\tau)$, where τ is a parameter that controls the absolute probability of infection. For fixed values of τ, the function $P(x;\tau)$ should take value zero when $x = 0$ (no exposure = no chance of infection), and if $x1 \leq x2$ then $P(x1;\tau) \leq P(x2;\tau)$ (an equal or greater magnitude of exposure should not decrease the probability of infection). The assumption of independent infection outcomes over multiple exposure events then leads to a simple expression for the probability of infection over a year follow-up period, given by $Pr(M.\ tuberculosis\ \text{infection}) = 1 - E\{E[1 - P(X;\tau)]\}^M$, where E represents expectations taken over the assumed distributions for X and M. One specific form for $P(X{:}\tau)$ is given by $1 - (1 - \tau)^X$, which is motivated by assuming that each bacillus has an independent probability τ of forming a persistent infection at the site. Although this functional form is motivated by a specific biological model, it provides a broad range of shapes for $P(X;\tau)$ (Fig. 2A) and is reasonable to consider on that basis even without reference to the specific biological model. The impact of innate immunity, host genetics, vitamin D levels, or other factors that may influence the probability of an infectious quantum establishing a persistent focus of infection following deposition in the lung is contained within the assumptions of the model as an aggregate host risk factor. A more nuanced incorporation of such factors into the model is not feasible with currently available data.

In summary, our model linking exposure intensity and magni-

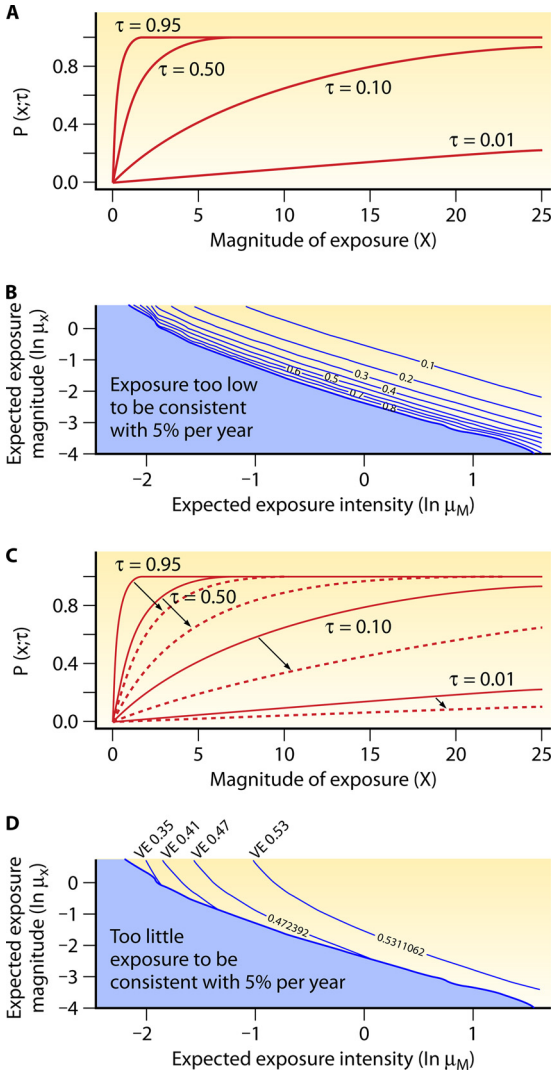

FIG 2 Mathematical modeling of effects of a preexposure TB vaccine. A family of models, indexed by a parameter τ, which translates an exposure event with exposure magnitude X to a probability that the exposure will lead to a persistent *M. tuberculosis* infection (τ controls the absolute probability of infection), is shown. The average of $P(X;\tau)$ over the assumed distribution of X gives the unconditional probability of persistent *M. tuberculosis* infection for a single exposure. Terms are as follows. M is the number of discrete exposure events over a year (intensity of exposure). Assume that M varies over individuals but there is an average number of exposure events for a given population of individuals and that average (on log scale) is the index of intensity of exposure for that population. X is the infectious potential of a single exposure event (magnitude of exposure). The simplest interpretation of the infectious potential X is as the number of discrete infectious units (e.g., bacilli) deposited on the lung alveolar surface at a single exposure event. Even though X can be interpreted more generally/abstractly, the narrow interpretation of X is the number of infectious units per exposure event. Assume that X varies over multiple exposure events within individuals as well as between individuals but there is an average number for a given population of individuals over time and that average (on log scale) is the index of magnitude of exposure for that population. τ is a parameter that links exposure magnitude X to probability of infection through the function $P(X;\tau)$. For the specific function $P(X;\tau) = 1 - (1 - \tau)^X$, τ is the probability of infection from a single unit exposure (e.g., an exposure event with $X = 1$). (A) Probability of persistent *M. tuberculosis* infection in relation to different exposure magnitudes. The graph shows the probability of persistent infection for a given exposure magnitude (number of bacilli per exposure event). The magnitude of exposure is plotted on the x axis, where X may be interpreted as the number of discrete infectious units deposited in the lung for an individual exposure event. The y axis represents the index of infectiousness. Four different possible scenarios for probability of infection (τ) are plotted. (B) Model for different probabilities of infection calibrated to an annual infection rate of 5%. the contour plot shows the relationship between exposure magnitude and intensity corresponding to an annual infection rate of 5%. The range of potential exposure magnitudes is plotted on the x axis (log scale). The range of potential exposure intensities (number of exposure events) is plotted on the y axis. The contour lines indicate potential values for the probability of infection (τ) for given expected exposure magnitudes and intensities. The blue region represents values that are not consistent for a setting with an observed population infection rate of 5%. (C) Model for vaccine effect in which the probability of infection is reduced by 60%. The estimated effect of a vaccine with 60% biological efficacy (reduces the probability of persistent infection by 60%) is shown. Solid lines correspond to the scenarios depicted in panel A. Dashed lines correspond to reduced probability of infection expected for a vaccine with 60% efficacy, with arrows highlighting the amount of shift. The graph suggests that for a low probability of infection (τ), the vaccine effect is reduced (arrow). In addition, at high probabilities of infection, the vaccine effect is reduced at higher magnitudes of exposure. For lower probabilities of infection, the vaccine effect persists across a wide range of exposure magnitudes. However, for higher probabilities of infection, the vaccine effect is apparent only at lower exposure magnitudes and is almost completely attenuated at higher exposure magnitudes. (D) Attenuation of vaccine efficacy for different levels of magnitude and intensity of exposure. Contours of values for vaccine efficacy (VE) plotted versus exposure intensity and magnitude are given for a 60% reduction in the probability τ (RR = 0.4). The contour plot shows values for biological vaccine efficacy calibrated to an incidence of 5% per year. This graph is a companion to panel B, in which the per-exposure probability of infection (τ) is consistent with population infection rates of 5% per year for certain levels of magnitude and exposure. The contour line numbers indicate different potential population-level or observed vaccine efficacies associated with a vaccine with biological (per-exposure) efficacy of 60%. As in panel B, the blue region indicates scenarios in which exposure is too low to be consistent with an unvaccinated population infection rate of 5% per year. The model suggests that observable population-level vaccine efficacy decreases as exposure decreases and the per-exposure probability of infection concomitantly increases. It also suggests that attenuation of vaccine efficacy is greater for high-magnitude/low-intensity exposure profiles than for low-magnitude/high-intensity profiles. Thus, all other things being equal, a vaccine would perform better with more exposures of lower magnitude than with fewer exposures of higher magnitude.

tude to infection posits M exposures per year with exposure intensities X, where the probability of infection from a given exposure depends on the intensity of that exposure X together with the parameter τ, which can be interpreted as the probability of infection given exposure to a single bacillus.

Suppose that the annual rate of *M. tuberculosis* infection in a given high-burden population is approximately 5%. Thus, for a given degree of intensity and magnitude of exposure, one can compute what the value of τ must be in order to match that known rate of infection. Simply put, if exposure intensity and/or magnitude is much higher than the observed infection rates, then the probability of infection per exposure to a single bacillus must be quite small. Conversely, if exposure is low relative to observed infection rates, almost every exposure must lead to infection. There is a threshold or exposure below which it is impossible to achieve a given infection rate even if exposure leads to infection with certainty. The result of this computation calibrates the model to a given annual infection rate given the assumed degree of exposure. Figure 2B displays contours of the value of τ calibrated to an annual infection rate of 5% across different combinations of intensity and magnitude of exposure (indexed values of μ_X and μ_M on the natural log scale). This figure shows one region in blue for which levels of exposure are too low to be consistent with an annual infection rate of 5%. The boundary of this region corresponds to the deterministic situation for which every exposure

results in a persistent *M. tuberculosis* infection ($\tau = 1$). Higher levels of exposure correspond to the stochastic situation for which each exposure does not inevitably result in persistent infection ($\tau < 1$).

Modeling observed VE. With the model calibrated to an annual infection rate of 5%, we can then explore how a reduction in the probability τ by prior vaccination would translate into observed vaccine efficacy (VE) and how variations in the intensity and magnitude of exposure might affect that vaccine efficacy parameter. We define the population-level vaccine efficacy as the percent reduction in the annual infection probability and compute it from the expression given above using the values of τ represented in Fig. 2A to calibrate the control group infection rate at 5% and using values of τ * RR to give the corresponding infection rate among vaccinees, where RR is the relative reduction in the probability τ due to prior vaccination. One minus RR might be thought of as "biological vaccine efficacy" at the level of a single exposing bacillus. Examples of the assumed shift in the function $P(X;\tau)$ due to prior vaccination (with 60% reduction in the probability τ) are shown in Fig. 2C.

Contours of values for vaccine efficacy (VE) plotted versus exposure intensity and magnitude are given in Fig. 2D for a 60% reduction in the probability τ (RR = 0.4). As expected, the value of population-level vaccine efficacy is attenuated relative to the "biological vaccine efficacy." The magnitude of attenuation is greatest for lower levels of exposure at the threshold of a deterministic link between each exposure event and infection. Thus, if exposure is driving infection with little biological variation in risk subsequent to exposure (i.e., $\tau \approx 1$), then vaccine efficacy is more likely to be significantly attenuated. In addition, the degree of attenuation appears to be more sensitive to intensity rather than magnitude of exposure, with the combination of low intensity and high magnitude resulting in vaccine efficacy of nearly one-half the value of τ, while high intensity and low magnitude results in vaccine efficacy of only 22% less than the value of τ. The result of this simple modeling exercise supports the idea that variation in exposure would not dilute a biological effect of preexposure vaccination to a degree that population-level vaccine efficacy could not be reliably detected in a clinical trial.

Summary of modeling exercise and conclusions. To recap in simple prose, suppose a vaccine has a "biological efficacy" of reducing the probability of infection per single unit exposure by 60%. The question is how much of a reduction in population rates of infection such a vaccine would produce given the multiplicity of exposures over time and variation in the magnitude of exposures (over different exposure events). For a given intensity and magnitude of exposure in a population, we can model the rate of infection as a function of infection probability from a single unit exposure. For a known infection rate in a specific population, e.g., 5%, we can then calibrate the model to compute the infection probability per single unit exposure that corresponds to the population infection rate under the specified intensity and magnitude of exposure. If the exposure is at very high levels relative to the population infection rate, then the infection probability per single unit exposure must be small. If exposures are at very low levels, then the infection probability per single unit exposure must be high, and at some point it must approach one, where every exposure results in infection. With this model calibrated to a population infection rate in an unvaccinated population, we can compute the population infection rate among vaccinees, assuming a given level of biological efficacy of the vaccine. From these population rates we can compute observed vaccine efficacy and compare it to biological vaccine efficacy to see how much of that biological efficacy is attenuated due to assumed exposure intensity and magnitude. What we find is that biological vaccine efficacy decreases when exposure magnitude and intensity decrease, with the lowest efficacy when approaching the threshold at which the probability of infection per single unit exposure approaches 1 (i.e., infection occurs after every exposure). However, provided that there is some stochasticity in infection per given exposure (i.e., exposure does not inevitably result in infection), then attenuation of biological vaccine efficacy is not that great and estimates of the observed vaccine efficacy in a vaccine trial may reasonably reflect the levels of biological efficacy of the vaccine.

Previous modeling studies suggest that the protective effects of a "leaky vaccine" are imperfect and are realized independently over multiple exposures (165) The overall protective effect of the vaccine declines, and measurable vaccine efficacy is much less than that realized in a population of individuals with few exposures over time. The contribution of the modeling work here is to calibrate the model to a fixed population-level rate of infection (e.g., 5%) and note that increased levels of exposure in this calibrated model naturally must be offset by a reduction in the absolute probability of infection per exposure. The novel finding of this work is that, once the model is calibrated, the attenuation of protective effects of a vaccine is increased when there is less exposure and the probability of infection per exposure is close to one. Thus, the general concern about protective effects of a TB vaccine being overwhelmed by multiple exposure events is perhaps misplaced. A second result of this modeling has to do with the relative impact of heterogeneity in magnitude of exposure compared to intensity of exposure over time. Again, fewer exposures that are each of a very high magnitude will erode the protective effects of a TB vaccine more than greater numbers of exposures that are each at a low magnitude.

A PROGRAM FOR TB VACCINE DEVELOPMENT

Characteristics of Prevention-of-Infection and Prevention-of-Disease Trials

Licensure of a TB vaccine will ultimately hinge on direct demonstration of the clinical benefit of vaccination and thus will require randomized vaccine efficacy trials with primary clinical endpoints of morbidity and mortality associated with active TB disease. Such trials would be extraordinarily costly due to the large sample size (tens of thousands of participants) and long duration of follow-up (at least 5 years) needed to observe the required number of TB disease endpoints within a trial cohort that is uninfected at enrollment. The investment required to perform such an expensive trial would be warranted if it was highly plausible that the vaccine candidate was efficacious. However, the lack of clear immune correlates of protection weakens the interpretation of immunogenicity data regarding plausible vaccine efficacy, and animal challenge models have generally been poor predictors of vaccine performance in humans. Moreover, nonpediatric TB vaccine efficacy trial designs that have been proposed (e.g., by Rustomjee et al.) are powered almost exclusively on detecting effects among subcohorts with postexposure vaccination (166). Thus, it is not surprising that pivotal trials of vaccine candidates employed in a preexposure vaccination strategy have not been mounted until now. A

phase II proof-of-concept trial of prevention of TB infection recently commenced among healthy, HIV-uninfected, previously BCG-vaccinated adolescents near Cape Town, South Africa, an area with a very high force of infection in this age group (ClinicalTrials.gov registration no. NCT02075203) (17, 23). This clinical trial will test both BCG revaccination and the adjuvanted protein vaccine H4-IC31 (AERAS-404), each compared to placebo, for safety, immunogenicity, and protection against TB infection ($n = 990$) as measured by QFT-GIT conversion. Protection against infection, measured by persistent QFT-GIT conversion without subsequent reversion through 6 months after initial conversion, will also be evaluated.

As described above in TB Epidemiology in High-Prevalence Settings, the rate of TB infection among adolescents is much higher than that of TB disease, so trials to evaluate preexposure vaccines for reduction in the rate of TB infection would be much smaller and shorter in duration than those with TB disease endpoints. The demonstration of a substantial reduction in the rate of TB infection would certainly be an important marker of a vaccine's biological activity and would provide a strong argument for plausibility of vaccine efficacy to prevent TB disease. Moreover, lasting protection from infection could potentially interrupt the cycle of disease and transmission. However, TB infection should not be considered a surrogate endpoint to replace TB disease in pivotal trials as there is no guarantee that an infection prevented by prior vaccination would not simply be in one of the 90% of individuals who never progress from latent infection to active TB disease. Thus, trials with a TB infection endpoint should best be considered phase II trials that can deliver strong evidence for plausibility of clinical vaccine effects and form the basis for gating multiple vaccine candidates for advance to evaluation in pivotal trials with disease efficacy endpoints. Such trials represent a rational stepwise path leading to phase III trials of preexposure TB vaccines.

Vaccine Design from Preclinical to Clinical Development

The present preclinical development is focusing on the use of the older "Riley" model, in which it appears there may be transient infection in guinea pigs exposed downstream of infected human subjects (93). In addition, the present NHP model in use starting in 2014 by Aeras delivers an inoculum 1 to 5 CFU, so prevention of actual infection may become measurable and desirable, as this model may better approximate true human infection. Initial work by us (data not shown) has also resulted in controlled NHP-to-NHP transmission via the aerosol route, which also holds promise to evaluate vaccines for prevention of infection.

As these preclinical models for novel vaccine candidates improve, selecting the actual vaccine to move into human prevention-of-infection studies may become more evident. At present, given a lack of convincing preclinical data, the decision to move specific candidates forward has been made based on diversity, (such as whole mycobacterial versus subunits in a trial comparing BCG and the IC-31-adjuvanted fusion protein hybrid 4), some level of animal data indicating a degree of "low take" based on immunological responses (the IC-31-adjuvanted protein H56), and knowledge based on antigens expressed during the early stages of infection ("acute-phase antigens"). Studies are also ongoing to determine whether specific innate signatures may be associated with potential prevention of infection (for example, in uninfected, highly exposed household contacts) and then to examine what vaccine strategies might recapitulate the induction of such gene signatures. The pipeline of available clinical approved (phase I or higher) vaccine products has expanded considerably over the past 10 years (Table 1) but mainly favors protein antigens. One limitation of the current pipeline is the number of products that contain the same antigens (which are mostly immunodominant) and/or similar adjuvants.

Longer-term strategies could include research areas beyond these peptide-restricted T-cell responses. For example, lipid antigens that are targets of CD1-restricted T cells could provide a distinct immune response (167). Targeting humoral immune responses, a successful strategy for most of the currently available vaccines, has not been a priority area of research in TB immunology. Antibodies offer a specific conceptual advantage for a preinfection vaccine since they have the potential to prevent binding and uptake of *M. tuberculosis* by a macrophage. Furthermore, several lines of evidence suggest that *M. tuberculosis*-specific antibodies may contribute protective responses to *M. tuberculosis* (168–172). Although other studies suggest that B cells do not mediate protection in murine TB models, further research is needed to determine the role of B cells in human TB pathogenesis (173, 174). Research in this area could lead to a broader immunological landscape that can be sampled and tested in vaccine products. Testing of strategies that result in high levels of effector cells in the lungs, such as the use of cytomegalovirus (CMV) vectors and aerosolized viral vectors such as modified vaccinia virus Ankara (MVA) and adenoviruses, is also under way. Although these strategies are likely to result in an "aborted" infection, the present tools available to measure responses in human trials would not be able to necessarily distinguish between a strategy that blocked infection of the macrophage and one that resulted in a limited T-cell response resulting in a negative TST or IGRA result. It is clear that focused future studies such as those mentioned here are needed in the area of animal models, vaccine design, and new methods to determine at what stage "infection prevention" is actually occurring.

Infection endpoint trials that demonstrate vaccine efficacy will provide opportunities to identify correlates of protection against *M. tuberculosis* infection as well as mechanisms underlying such protective immunity. In light of the current lack of correlates of protection against TB disease and *M. tuberculosis* infection, studies of correlates should be an important and valuable component of infection endpoint trial design. The smaller sample size relative to that for TB disease endpoint efficacy trials may allow collection of comprehensive specimen sets at more follow-up time points. Further, frequent IGRA testing during follow-up would allow early detection of new *M. tuberculosis* infections, facilitating studies of the poorly understood biology underlying acute *M. tuberculosis* infection of humans.

Clinical Trial Design, Endpoint Definitions, and Issues

A principal challenge in the design of an infection endpoint trial is the accurate assessment of prevalent infections at baseline and incident infections during follow-up. In addition, the durability of protection from infection will be a critical factor determining whether an impact could be observed at the population level. Unfortunately, there is no microbiological assay that can directly measure the *M. tuberculosis* bacillary burden in tissues that are readily and repeatedly sampled in large trial cohorts during the paucibacillary early stages of infection. Instead, the use of *M. tuberculosis*-specific immunological assays is the only viable option,

such as TST and IGRAs (T-SPOT and QFT-TB Gold). The precise sensitivity and specificity of these assays as used for infection endpoints and baseline screening for prevalent infections are uncertain because of the lack of a true gold-standard measure of infection and uncertainties in the dynamics of the cellular immune response to infection. The operating characteristics for these assays were developed for settings in which the infection is not endemic and may require additional assessment before use in a vaccine trial in a setting with endemicity. In addition, the current IGRAs are all focused on IFN-γ measurements with a limited dynamic range. Therefore, it may be beneficial to move beyond pure reliance on IFN-γ to identify assays with a larger dynamic range. The potential for tuning the parameters of the assays (e.g., specific threshold values for positivity calls and replicate assays to minimize technical variation) will need to be carefully considered, although without a gold-standard assay these exercises will necessarily be driven by judgment calls rather than precisely measured operating characteristics.

Screening at baseline to exclude subjects with prevalent infections from enrollment will necessarily miss those infections so recent that measureable cellular responses have yet to mature. If vaccination after infection is not protective, then inclusion of the subset of recent prevalent infections in the trial cohort can attenuate the observed vaccine efficacy and degrade statistical power. The kinetics of cellular responses in immunocompetent individuals suggests that analyses of endpoints occurring at least 2 to 3 months from baseline will provide the time to wash out the effects of the prevalent infections and recover an accurate estimate of vaccine efficacy specific to preexposure vaccination.

The phenomenon of reversion occurs in a small but important fraction of IGRA converters in whom measured cellular responses above the threshold for positivity decline to levels below that threshold. It is unclear whether this phenomenon represents a host-side immunological defect (i.e., in the durability of cellular responses to *M. tuberculosis*) or evolution of the infection to a state that is so immunologically silent that the cellular response contracts to immeasurable levels. The latter may be due to a host-side immunological success in clearance of infection or to a pathogen-side success in establishing immunologically silent latent infection (as discussed above in Biological and Immunological Intervention Points from Exposure to Infection). It is important for a trial to capture information about reversion as an important secondary endpoint defining vaccine efficacy and as a biomarker to provide insight into possible mechanisms of vaccine action. The definition of a secondary trial endpoint capturing "sustained conversion" provides one approach to dealing with the phenomenon of reversion. Sustained conversion might be operationally captured by initiating a more intense follow-up schedule for study subjects who have a primary conversion endpoint, during which a longitudinal series of assays would be performed to document the persistence of conversion for some predefined period of time. This approach would result in the identification of a subset of the primary endpoints that represent sustained converters, and the impact of vaccination on the relative rate of occurrence for this more stringent endpoint would be defined. Exploratory analyses could further examine the impact of vaccination on the rate of reversion (among converters in vaccine and placebo groups); however, this analysis would not have the rigor of a fully randomized comparison, as it is based on a subgroup defined postrandomization. An important ethical consideration in the endpoint selection is the recommendation for INH treatment for QFT conversions. Given that recently infected individuals are in a high-risk group for developing active TB, there should potentially be a recommendation to treat with INH for LTBI after that endpoint is reached. Such a recommendation may not be appropriate in countries with high TB burdens among populations at high risk for reinfection (175). Capturing the "sustained conversion" endpoint (requiring 2 consecutive positive QFTs to deal with the possibility of false positives) would likely be ethical if the period between tests is short enough to minimize the risk of developing active TB (e.g., 1 month).

In some situations, endpoint assays are known to have imperfect sensitivity and specificity relative to a gold-standard clinical endpoint, and vaccine efficacy is defined based on the underlying true clinical endpoint. In these cases, the attenuation of vaccine efficacy by use of the imperfect endpoint assay in a trial can be defined (176). Trial design exercises can then account for this attenuation by increasing sample size to ensure power to detect levels of true vaccine efficacy based on the smaller levels of efficacy that can be observed in the trial. It is natural to think of applying this approach to the design of infection endpoint trials for *M. tuberculosis*; however, its application should be carefully considered. The lack of a gold-standard clinical endpoint definition and the resulting imprecision in characterizing endpoint assay sensitivity and specificity argue against this approach.

Another issue in the design of infection endpoint trials relates to the trial objectives and balance of risks for false-positive and false-negative outcomes. In most standard trial designs, the risk of a false-positive outcome is paramount, and careful attention is paid to control of "alpha" to very low levels (e.g., 0.025 1-sided). Relatively less concern is given to false-negative outcomes, where sample size is often determined given fixed "alpha" as a trade-off between trial cost, logistics, and risk for false-negative outcomes (e.g., 0.20) at some fixed important level of vaccine efficacy. As described above, the motivation of an infection endpoint trial is one of up-selection of candidate vaccines, and as such, the balance between risks of false-positive and false-negative outcomes is arguably more balanced than is usual. Thus, for such trials it is reasonable to consider designs that have somewhat larger-than-standard false-positive rates and somewhat lower-than-standard false-negative rates (e.g., both at 0.05 or even 0.10). For endpoint-driven trial designs to distinguish VE of 60% versus 0%, if performed in South African adolescent populations, would require sample sizes in the range of 1,000 to 3,000 and followed for less than 2 years.

Implementation

If an efficacious prevention-of-infection vaccine is developed, several implementation issues would need to be considered. First, would such a vaccine replace BCG or be used in a prime-boost strategy (BCG prime with boost from the new vaccine)? Prime-boost combinations would need to be carefully evaluated due to the possibility of BCG priming a variety of immune responses that could be beneficial or deleterious. Second, replacement of BCG with a new vaccine would pose implementation challenges and might require substantial changes in population attitudes as well as the health care delivery system to be successful.

CONCLUSION

With focused attention on *M. tuberculosis* as an infection endpoint, the benefits of smaller sample sizes in efficacy trials would enable a rational stepwise vaccine research agenda that culminates in trials with TB disease as the endpoint. The challenges are many and include prioritization of vaccine products and selection of endpoint assays, endpoint definitions, sample sizes, and target populations. Although there are many challenges to be solved for successful development of a preexposure TB vaccine, there are also numerous opportunities.

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